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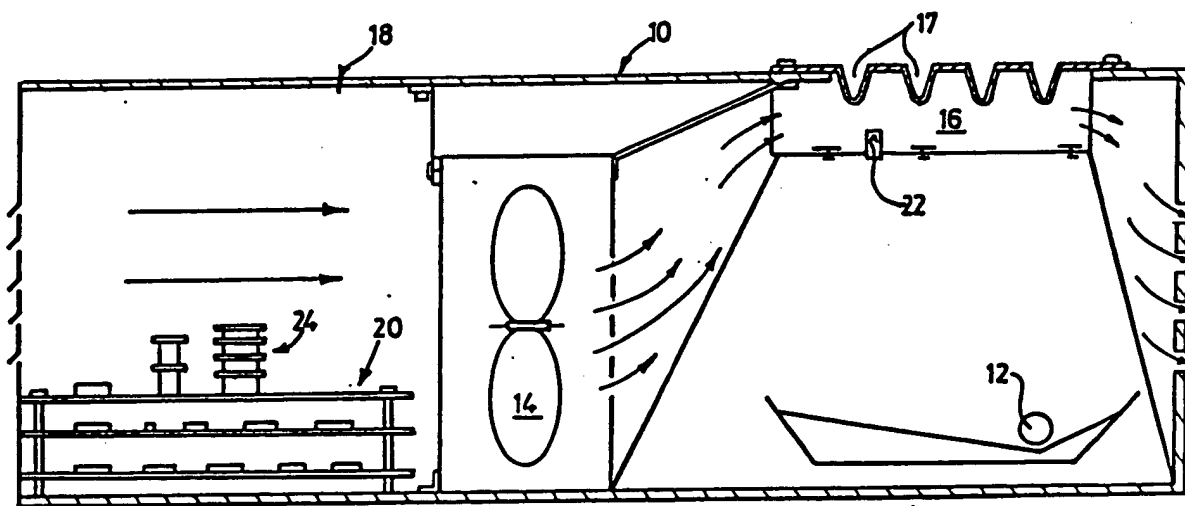
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(54) Title: REACTION TEMPERATURE CONTROL



(57) Abstract

Apparatus for controlling the temperature of a reaction so that it follows a required temperature/time profile, wherein a multi-well support (16) having a plurality of reaction sites (18) is heated by a quartz-halogen lamp (12) and cooled by a fan (14), the switching on and off of the lamp and the fan being controlled by relays controlled by a programmable microprocessor (48) which receives input signals from at least one reaction-temperature sensor (38).

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Title: Reaction Temperature Control

Field of invention

This invention relates to apparatus for and a method of controlling the temperature of a reaction zone. The invention is primarily concerned with controlling the temperature of biological reactions, but is not restricted to such reactions and for simplicity details of specific biological or chemical reactions are not specifically referred to in the ensuing description or the appended claims. However, biological and chemical reactions when controlled in accordance with the invention are not to be considered excluded from the scope of the invention.

Background to the invention

Many biological and molecular biological reactions require incubation at differing temperatures for different time periods. Heating blocks and/or water baths are conventionally used as reaction zones but such are unable to change reaction temperatures rapidly, and in some circumstances are therefore unable accurately to control a reaction in accordance with a required temperature profile. Indeed it may not even prove possible accurately to maintain a truly constant reaction temperature, if this is the profile required.

It is an object of this invention to provide an improved system of reaction zone temperature control.

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Summary of the invention

According to one aspect of the invention, apparatus for controlling the temperature of a reaction zone contained within a support therefor comprises a source of radiant energy, a fan, reaction temperature sensing means, a microprocessor responsive to the output of the temperature sensing means, and control means responsive to output signals from the microprocessor to control the operation of at least one of the source and the fan in order to conform the reaction zone temperature to a required temperature/time profile.

According to another aspect of the invention, there is provided a method of controlling the temperature of a reaction zone, according to which the reaction zone temperature is sensed by at least one temperature sensor, and the temperature/time profile of the reaction zone is achieved using a source of radiant energy for raising the temperature and a fan-induced flow of air for reducing the temperature, and on/off switching of the source and of the fan are independently controlled by a control means itself controlled by a programmed microprocessor supplied with signals from the reaction zone temperature sensor.

The source of radiant energy may be a lamp.

The apparatus and method above described are especially useful for temperature control of biological reactions as a polymerase chain reaction for DNA amplification or an enzyme mediated DNA modification reaction but the invention may be employed for any reaction zone temperature control whether the reaction to one of the

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biological or molecular biological or general chemical natur .

For the purpose of biological reactions such as given above, it is convenient to use a support, which comprises a copper or other metal plate of small thermal capacity and high thermal conductivity, acting evenly to distribute heat derived from the source to at least one reaction site in the form of a shallow well on the support, where more than one reaction site is required a corresponding number of wells may be provided.

The wells may be formed as cups in the metal plate itself, so that reaction tubes such as "Eppendorf" tubes can be fitted into the cups.

Alternatively, the wells may be embedded in a tray, for example of plastics material, which seats on the metal plate, the metal plate being provided with through apertures to accommodate the wells.

Alternatively at least the bases of the wells may be transparent and if supported by a metal plate the latter is apertured to allow the wells to protrude downwardly therethrough.

In such an arrangement, the radiation can be arranged to be incident directly on the underside of the transparent wells, thereby to heat the reactants directly. An apertured shade may be provided, movable to adjust the amount of direct radiation received by the reactants. When maximum heating is required, direct radiation may be maximised. For lesser heating direct radiation may be excluded, and reliance placed on heating by conductivity

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through the shade. To this end the shade is preferably formed from a thermally conductive material such as copper or other thermally conductive material and be slidably located directly against the metal plate.

Where the wells are supported by an apertured metal plate the underside of the wells and the apertured plate conveniently occupy the same plane and the shade is a second apertured plate of metal in sliding contact with the underside of the apertured metal plate.

In a preferred system, the support, the source and the fan are accommodated in a housing having an air inlet and an air outlet. The source is located within the housing, and is preferably housed at the base of a reflective shield acting to direct radiation from the source toward the support for the reactants. The shield is preferably closed at the end adjacent the support by a transparent plate typically of a ceramic material. In combination with the shield, further air flow directing means in the housing ensure that the fan, when operable, causes a turbulent stream of cooling air to pass in contact with the support, in passage from the housing inlet to the housing outlet.

The source is preferably a quartz-halogen lamp emitting both visible and infra-red radiation.

The sensor may sense the temperature of the support and/or that of one or more reaction sites, the microprocessor being programmed to anticipate the time lag between the support temperature and the reaction temperature if only the support is sensed.

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Further features of the apparatus and method in accordance with the invention will be apparent from the following description, which makes reference to the accompanying drawings.

In the drawings:

Figures 1 and 2 are plan and elevational views showing the principle of the invention;

Figure 3 is a side elevational view of one arrangement, as though seen through a transparent side wall of a housing;

Figure 4 is a side elevational view of a second arrangement, similarly seen with Figure 4(a) showing a detail;

Figure 5 is a plan view of the arrangement of Figure 2, as seen from the underside of the housing lid;

Figure 6 shows another arrangement in similar view to Figure 3;

Figure 7 shows graphically a temperature profile, and

Figure 8 is a block circuit diagram.

Detailed description of the drawings

Referring to Figures 1 and 2, a main housing 10 accommodates a quartz-halogen lamp 12, a fan 14 and a metal support 16 for reactants, for example contained in reaction tubes seated in wells 17 in the support plate. To the side of the main housing 10 is a sub-housing 18

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accommodating a programmable microprocessor and associated electronics on printed circuit boards 20. Associated with the support 16 is a reaction temperature sensor 22.

In use, the operation of the lamp 12 and fan 14 are controlled by suitable control means such as isolating relays 24 fed with control signals from the microprocessor, which is programmed to respond to signals received from the reaction temperature sensor 22 in order to cause the reaction temperature of the reaction tubes carried by the support 16 to follow a required temperature/time profile.

Referring now to Figure 3, there is shown one embodiment of the invention wherein, for convenience, the same reference numerals as in Figures 1 and 2 are employed for similar parts.

The support 16 comprise a plate of copper or other thermally conductive metal of low thermal capacity and good thermal conductivity. The metal plate 16 is formed with twenty four cups or wells 17 in a 6 by 4 array, shaped closely to receive the lower ends of sealed Eppendorf reaction tubes 25. Above the plate 16, the tubes 25 are laterally supported by apertures in a block 27 of thermally insulating material such as polyethylene which is let into the top wall of the housing 10.

The quartz-halogen lamp 12 is accommodated at the bottom of a shield 28 of aluminium, with polished internal walls, whereby radiation from the lamp is concentrated on to the undersurface of the metal support plate 16.

A ramp surface 30 is provided in the housing 10, whereby when the fan is operating, the air is caused to flow in

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turbulent manner, as indicated by the arrow 31, past the support plate 16. Sub-housing 18 again accommodates the programmable microprocessor and associated electronics including isolating relays for switching the lamp and the fan on and off. The upper surface 32 of the sub-housing 18 is conveniently formed as a desk-top carrying a keypad for selecting the microprocessor program and a display unit such as an LCD display.

A reaction temperature sensor 22 is provided in the support plate 16, and if desired a second such sensor 22 may be provided in contact with one of the reaction tubes 25. Such a sensor will hereinafter be referred to as a "phial" sensor.

In use, switching on and off of the lamp and the fan is controlled by the microprocessor, responsively to signal from either one or both of the reaction temperature sensors, to produce a required temperature/time profile for the reaction taking place in the reaction tubes.

The insulation 27 reduces loss of heat from the support plate 16 and protects the operator against burning when handling the reaction tubes.

Referring now to the embodiment of Figures 4 and 5, the support here includes a thin plastics tray 33 of rectangular shape. The tray 16 is integrally formed with a column/row array of cups or wells 34 which may be designed to accommodate reaction tubes, such as "Eppendorf" reaction tubes, which may be formed from polypropylene or alternatively the tray 10 may constitute a welled reaction dish.

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The plastics tray 33 seals onto a thin thermally conductive copper plate 35 drilled or otherwise formed with through-apertures to accommodate and fit closely around the lower ends of the wells 34:

The two part support 33, 35 is mounted in the housing 10 just below an openable lid 36, hinged at 37 at one end to the housing and openable about the hinge 37 by means of a handle 38 at the other end, in order to give access to the support. The lid 24 is double-walled, and contains thermal insulation material 38, for example of sheet polypropylene. When the lid is closed, a relatively narrow space or passage 39 exists between the underside of the lid 24 and the support plate 16.

The quartz halogen lamp 12 is again mounted near the base of the housing 10 beneath the support, in operation to emit visible and infra-red radiation towards the underside of the support in order to effect heating thereof, and thereby heat reactants carried by the reaction tray 33.

The lamp 12 is mounted at the base of an upstanding aluminium shield 40 with polished interior surfaces 41 whereby loss of radiation from the lamp is minimised. The shield 40 is optionally closed at the top, just beneath the underside of the support 16, by a transparent ceramic plate 42. The lamp 12, shield 40 and ceramic plate 42 in combination define the shape of a truncated pyramid, base uppermost. A space or passage 43 exists between the ceramic plate 42 and the underside of the support 33, 35.

As illustrated, in particular in Figure 4(a), an apertured plate of copper 44 is mounted immediately beneath the support plate 35 to be slidably movable relative thereto,

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and with a layer of conductive grease therebetween. By relative sliding movement of the plate 44 and the plate 35 so as to align the apertures in the two plates, to a greater or lesser extent, so the reactant wells 34 will be exposed to or shaded from direct radiation from the lamp 12. To this end the apertured copper plate 42 is apertured in a row/column array corresponding to that of the reaction sites on the support.

In a preferred construction the support plate 16 comprises a 1mm thick copper sheet drilled or otherwise formed with ninety six apertures in which a corresponding number of reaction wells, (eg wells formed in a transparent polystyrene tray), are received. The bottoms of the wells are coplanar with the underside of the plate 35. The second copper sheet 44, which may be of similar thickness material, is drilled or otherwise apertured in like manner and is slidable directly beneath and in contact with the plate 35 so that the lower plate is in contact with the upper plate 35 for exposing more or less of the underside of each of the wells to the lamp radiation. In effect, this arrangement enables the liquid reactants to be directly exposed to the radiation, (through the transparent bases of the wells) whereby rapid heating to a relatively high temperature is possible. However, if only lower reaction temperatures are required the undersides of the wells may be shaded, by appropriate sliding of the second sheet so that heating of the reactants occurs partly or wholly by conduction. The second sheet may be manually adjustable or, as illustrated, adjustable by a motor 45 which drives a connecting rod 46 via a ratchet device 47. The motor 45 may also be employed to drive the fan 14, provided a suitable clutch or other disengagement means is provided.

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Where a very rapid rise in temperature is required the shading plate 44 is moved to fully expose the wells to the lamp radiation and as soon as the desired temperature is attained, is moved out of alignment to hold the temperature achieved.

Mounted in the housing 10 to the side of the lamp/shield assembly is the fan 14. In operation, the fan 14 draws in environmental air at room temperature through an air inlet, 48 in the top wall of the housing above the fan, and expels air through a louvred outlet 49 on the side of the lamp/shield assembly remote from said fan. Figure 5 shows the air holes 50 in the support plate mounting which enable the air to pass into and exit from the ends of the passage 39. The air is thus caused to pass in turbulent flow through the passages 39, 43 above and below the support 33, 35, thereby to effect cooling of the latter and the reactants carried thereby. Air flow within the housing is again generally indicated by the marked arrows.

Although not shown, the air inlet may alternatively be located on the remote side of the sub-housing.

The sub-housing 18 contains a microprocessor 51 and associated electronics on a stack 52 of printed circuit boards, and the upper surface 53 of the sub-housing again houses a keypad 54 and an LCD display 56 associated with the microprocessor.

The microprocessor 51 is able to receive signals output from either one or both of two reaction temperature sensors.

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The sensor 58 senses the reaction zone temperature indirectly by sensing the temperature of the plate 35.

The sensor 60 senses the reaction temperature directly, for example by dipping into the reactants in a selected sample reaction tube on the support plate.

The sensors 58, 60 may comprise thermocouples or thermistors of a commercially available kind having appropriate range and sensitivity for the purpose for which they are required.

The microprocessor 48 responds to the signals from one or the other (or both) of the two temperature sensors to provide switching signals for switching the lamp 12 and the fan 14 on and off, each independently of the other, in order to cause the temperature of the reactants on the support plate to follow a required temperature/time profile. This may for example be programmed into the memory of the microprocessor, for example by means of the keypad.

To enable it to follow the profile with respect to time, the microprocessor includes an electronic clock (not shown).

The required temperature profile may range from a constant temperature to a temperature varying between a plurality of upper and lower values with varying time intervals.

A temperature profile commonly required for biological reactions involves a temperature cyclically varying between two temperatures with a regular periodicity, as

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exemplified by Figure 4.

Figure 6 shows a modified arrangement in which the lamp/shield assembly 12, 40 is inverted and positioned above the support 16, which is accessible through a hatch in the side wall of the housing 10. The support 16 may be a welled upper plate as described with reference to Figure 3 or a composite support as described with reference to Figures 4 and 5, and is located on apertured guides which enable turbulent air flow beneath, as well as above the support. The housing surface beneath the support late 16 is reflectively coated. In this connection, it is mentioned that the underside of the lid in the embodiment of Figures 4 and 5 may also be reflectively coated.

Apart from the foregoing, the arrangement of Figure 6 is generally similar to that of Figures 4 and 5 and corresponding reference numerals are therefore employed.

Figure 5 is a block circuit diagram showing the control electronics, in which the same references as in Figures 1 to 3 are employed for corresponding components. Additionally, references 61 and 62 designate the ROM and the non-volatile RAM of the microprocessor 51, respectively, and references 64 and 66 denote an interface between the microprocessor 51 and the display 56 (which with isolators 68 in the form of solid state relays, may be a computer monitor screen), and between the microprocessor 51 and the keypad 54. The interface 66 also connects the microprocessor 48 with isolators 68 in the form of solid state relays, for effecting switching of the lamp 12 and the fan 14, and also with an alarm 70 such as a buzzer operative in the event of a fault. The inputs to the microprocessor 48 from the reaction-temperature

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sensors 58, 60 are supplied via conditioning amplifiers 72 and an analogue to digital converter 74.

Reed relays may be employed as the isolators 68.

In connection with the foregoing arrangements above described, the following further points of detail are to be noted:

1. In addition to being a good conductor having low thermal capacity, it is important that the support plate 16 should be well thermally insulated from its surroundings, to conserve the low thermal capacity and prevent heat leaks from surrounding parts of apparatus. A copper plate of about 1mm thickness will often be convenient.

2. The design of the polished reflective shield 34 to focus radiation from a tungsten halogen source onto the matt black base of copper plate is important, and the insulation of the top surface of the instrument also serves to prevent exposed hot surfaces.

3. The use of a "phial" thermocouple, ie direct-acting sensor 60, enables the temperature of a representative sample to be measured, giving more precise information than merely measuring the temperature of the plate.

If the temperature of the plate is controlled in a series of step changes, it will be seen that the temperature of the phial lags behind the plate temperature, reaching equilibrium much later. By using temperature information from the phial thermocouple 60, the controller can operate to produce an improved time response for the temperature

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profile of the sample, thus reducing cycle times.

The phial thermocouple 60 may also be used as a remote sensor. In this manner any existing manual technique which has been operated successfully, may be duplicated by first taking the "remote sensor" through the manual cycle, noting temperatures achieved, and then programming the IHB to automatically repeat this cycle.

4. The instrument is stand-alone, requiring only a single mains supply, and no fluids are involved.

5. A powerful, versatile programming protocol for the microprocesor 48 is desirable for giving either immediate constant temperature operation or a programmed cycle of temperature levels stored in non-volatile memory.

6. A suitable quartz-halogen lamp 12 is a conventionally available lamp of 300 to 500 Watts as commercially available, for example for floodlighting, whilst a suitable fan 14 is a Boxer (Trade Mark) electronic cooling fan, also commercially available.

7. Turbulent air flow is important for uniform cooling of the support plate 16, and this is substantially assisted by provision of the ramp for guiding the air flow into the passages 32, 40 above and below the support plate, as shown in the drawings.

8. The wells in the support plate 16 are desirably sufficiently spaced from the peripheral edge of the plate at least to minimise any edge effects.

9. An interlock is provided at the lid. As hitherto

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mentioned, a multiwell reaction dish may be fitted into the welled support plate. A pin on the lid prevents closing unless the multiwell dish is correctly positioned with its key at the correct corner. A microswitch, which operates upon contact with the multiwell dish, may also prevent operation unless a multiwell dish is in position. In the case of missuse or mid-cycle failure, operation will only recommence from the beginning after a reset.

10. One successful method for programming the temperature control is to use a polynomial equation to derive a relationship describing the "mark to space ratio" for the lamp flashing from the absolute required temperature and from the differential of the required temperature from the actual temperature. When large upward temperature jumps are required it is advisable to limit the differential term of the equation so as to avoid overshoot. This type of relatively simple control algorithm works well for predicting the plate temperature but in predicting the sample temperature, where there can be an appreciable lag in response (depending upon the sample volume or poor thermal conductivity in the case of a multiwell plate for instance), a time delay term becomes more important. There is a compromise to be struck between fast response time and the possibilities for oscillation. One simple way to provide a good sample control is to use a similar polynomial equation for the sample temperature and relate this not to the lamp flashing directly but to a setting for the plate temperature.

11. It is necessary to ensure optimal heat transfer between the support and the reaction tubes (or reaction dish). For ensuring this, the wells in the copper plate may be accurately shaped and dimensioned by

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electroforming, using a drilled copper sheet as a substrate and the reaction tubes themselves or the dish as a male mould. Alternatively, the wells may be formed by machining a solid block or by spinning.

In the event, it should be understood that, in comparison with reactant supports as have been employed in the prior art, the support plate employed in the present invention is of much lower bulk and much smaller thermal capacity and therefore possess a much smaller thermal inertia.

12. A modification from either of the two reactant supports hitherto described in a plain flat, thin copper support plate on which is freely placed a reaction dish of metal or plastics containing flat-bottomed cups or wells for reactants.

13. A prime purpose of the apparatus is to provide specified timed incubations for a designated enzyme reaction series as hitherto mentioned, with automatic control and auto-prompting of the operator.

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CLAIMS

1. Apparatus for controlling the temperature of a reaction zone contained within a support therefor comprises, a source of radiant energy, a fan, reaction temperature sensing means, a microprocessor responsive to the output of the temperature sensing means, and control means responsive to output signals from the microprocessor independently to control the operation of at least one of the source and the fan in order to conform the reaction temperature to a required temperature/time profile.
2. Apparatus according to claim 1, wherein the support comprises a metal plate of small thermal capacity and high thermal conductivity for acting evenly to distribute heat derived from the source to a plurality of reaction sites provided by a plurality of wells.
3. Apparatus according to claim 2, wherein the wells are provided in the upper surface of the metal plate.
4. Apparatus according to claim 2, wherein the support includes a tray of transparent plastics material preformed with wells, and the tray seats on the metal plate with the wells accommodated in through-apertures in the plate.
5. Apparatus according to any of claims 1 to 4, wherein the source is a quartz-halogen lamp housed at the base of a reflective shield acting to direct light from the lamp towards the support for the reactants, and the shield is closed at the end adjacent the support by a transparent ceramic plate.

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6. Apparatus according to any preceding claim, wherein the source is positioned beneath the support, whereby no direct radiation falls on the reactants.
7. Apparatus according to any of claims 1 to 5, wherein the source is positioned above the support, whereby radiation from the source falls directly on the reactants.
8. Apparatus according to any preceding claim, wherein the support, the lamp and the fan are accommodated in a housing having an air inlet and an air outlet, and the housing contains air flow directing means for causing a turbulent stream of air past the support when the fan is operating.
9. Apparatus according to claim 6, wherein the housing has a lid of thermally insulating material movable to provide access to the support.
10. Apparatus according to any of claims 1 to 9, including an openable and closable apertured shade positioned between the source and the support.
11. Apparatus according to any of claims 1 to 10, wherein the reaction temperature sensor means comprises a sensor which senses the temperature of the support, and a second sensor for sensing the temperature of the reactants at at least one of the reaction sites.
12. A method of controlling the temperature of a reaction zone, according to which the reaction zone temperature is sensed by at least one temperature sensor, and a

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temperature/time profile is determined by a source of radiation for raising, and a fan-induced flow of air for reducing, the zone temperature, and on/off switching of the source and of the fan is independently controlled by a control means itself controlled by a programmed microprocessor supplied with signals from the temperature sensor.

13. A method according to claim 12, applied to heat and cool the reaction in accordance with a required cyclic sequence of temperature variations.

14. A method according to claim 12 or claim 13, applied to control a polymerase chain reaction for DNA amplification, or an enzyme mediated DNA modification reaction.

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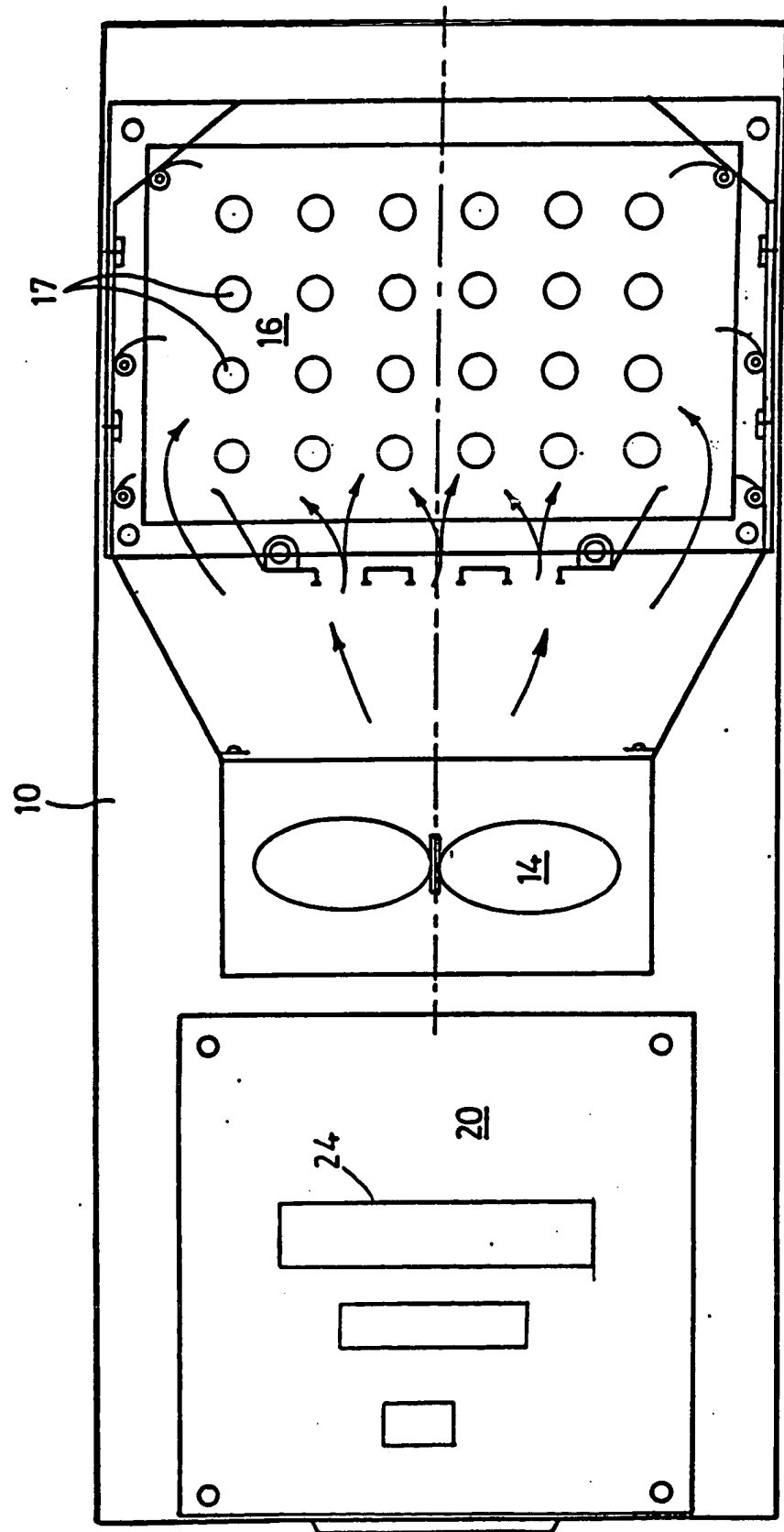


Figure 1

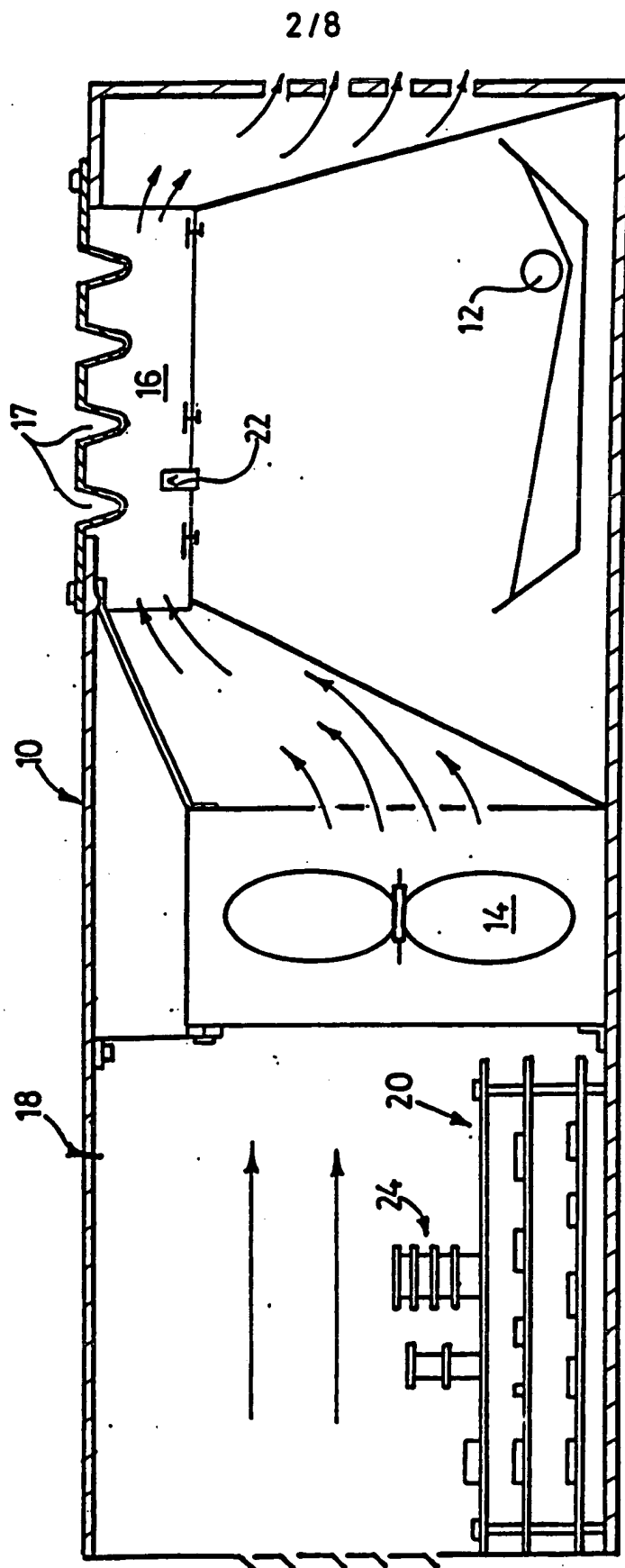


Figure 2

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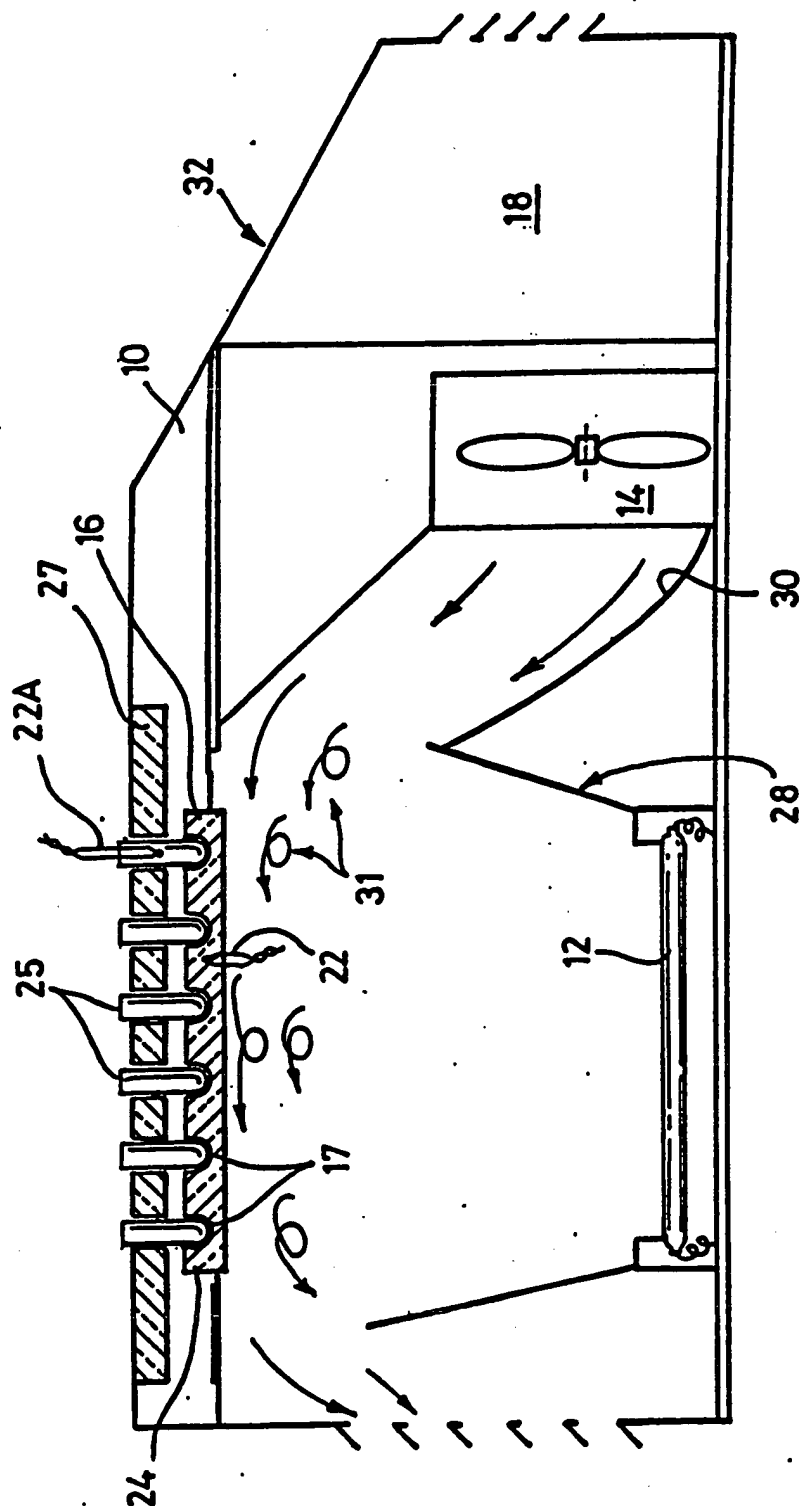
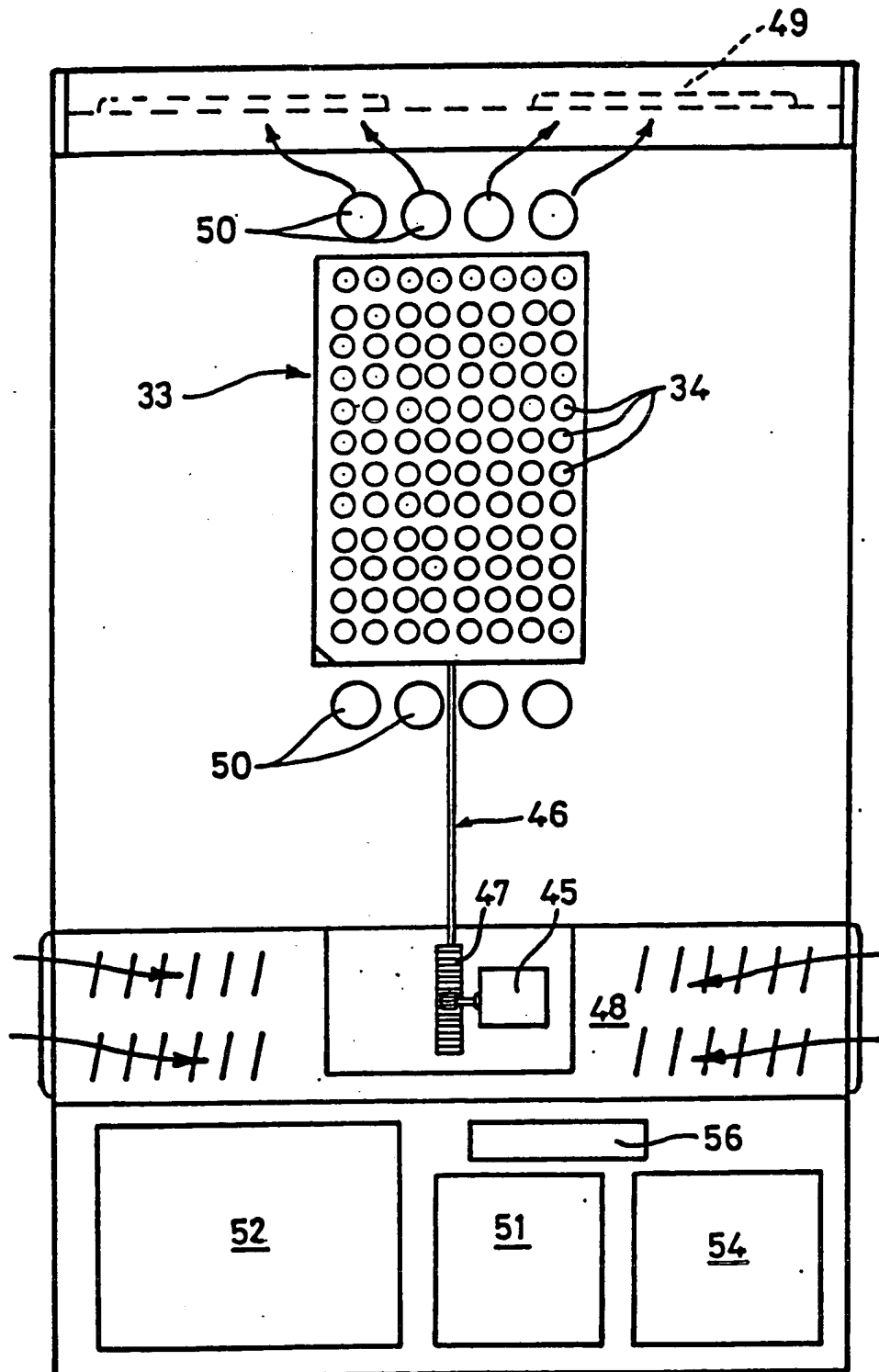


Figure 3

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*Figure 5*

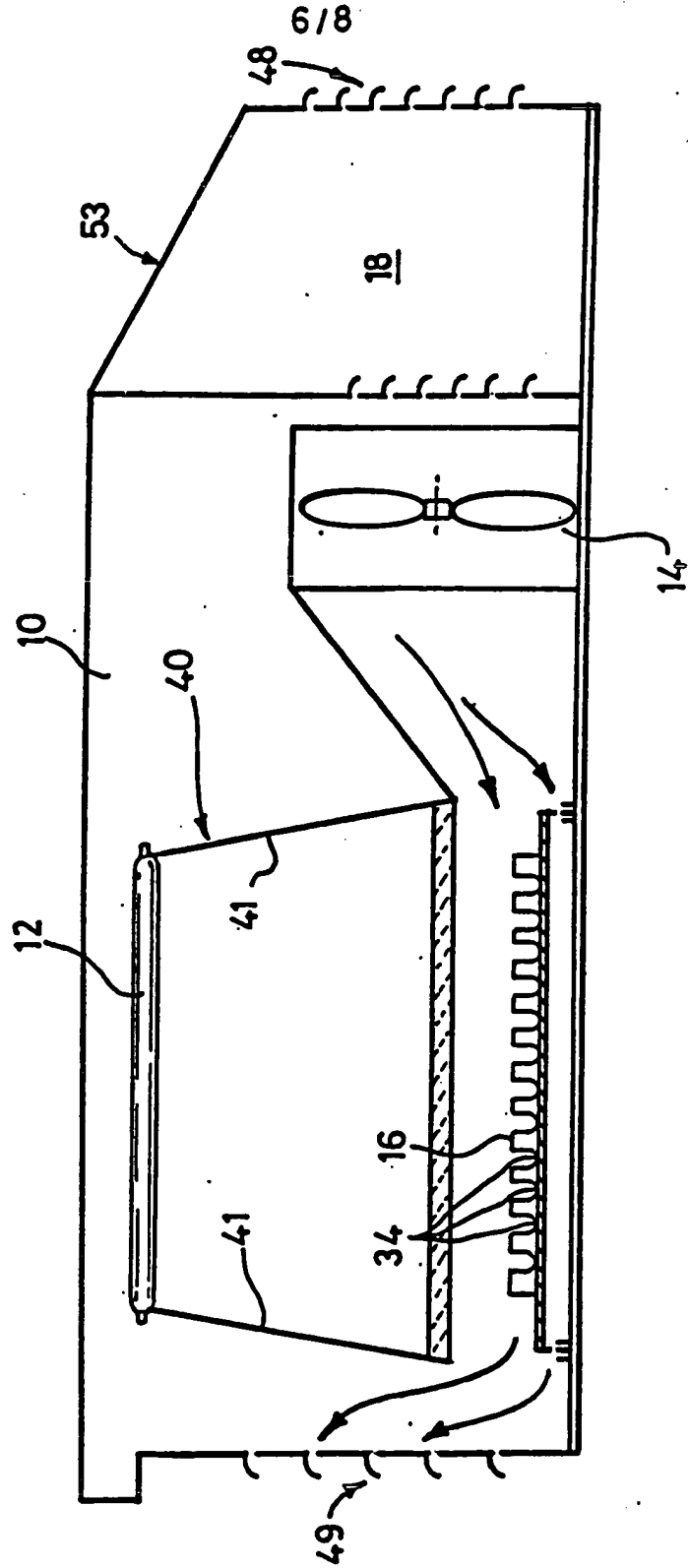
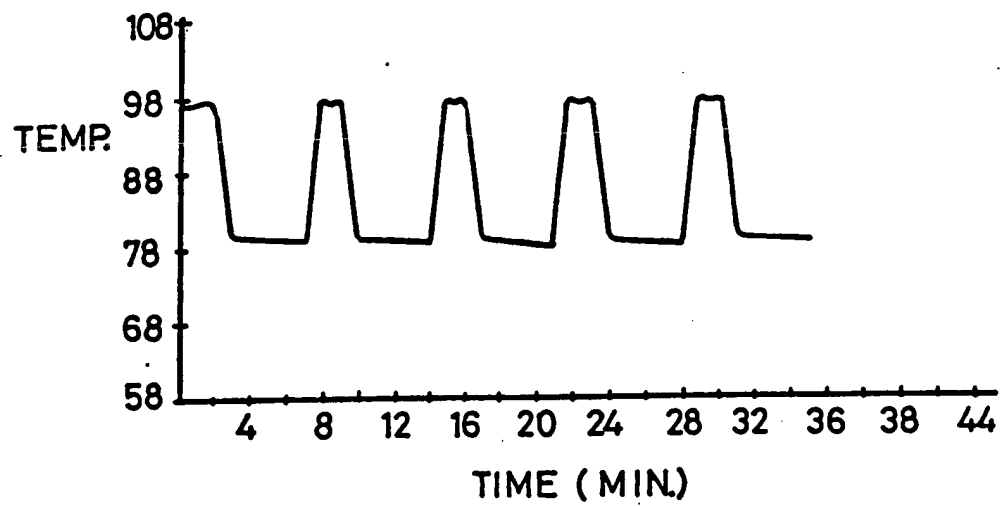


Figure 6

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*Figure 7*

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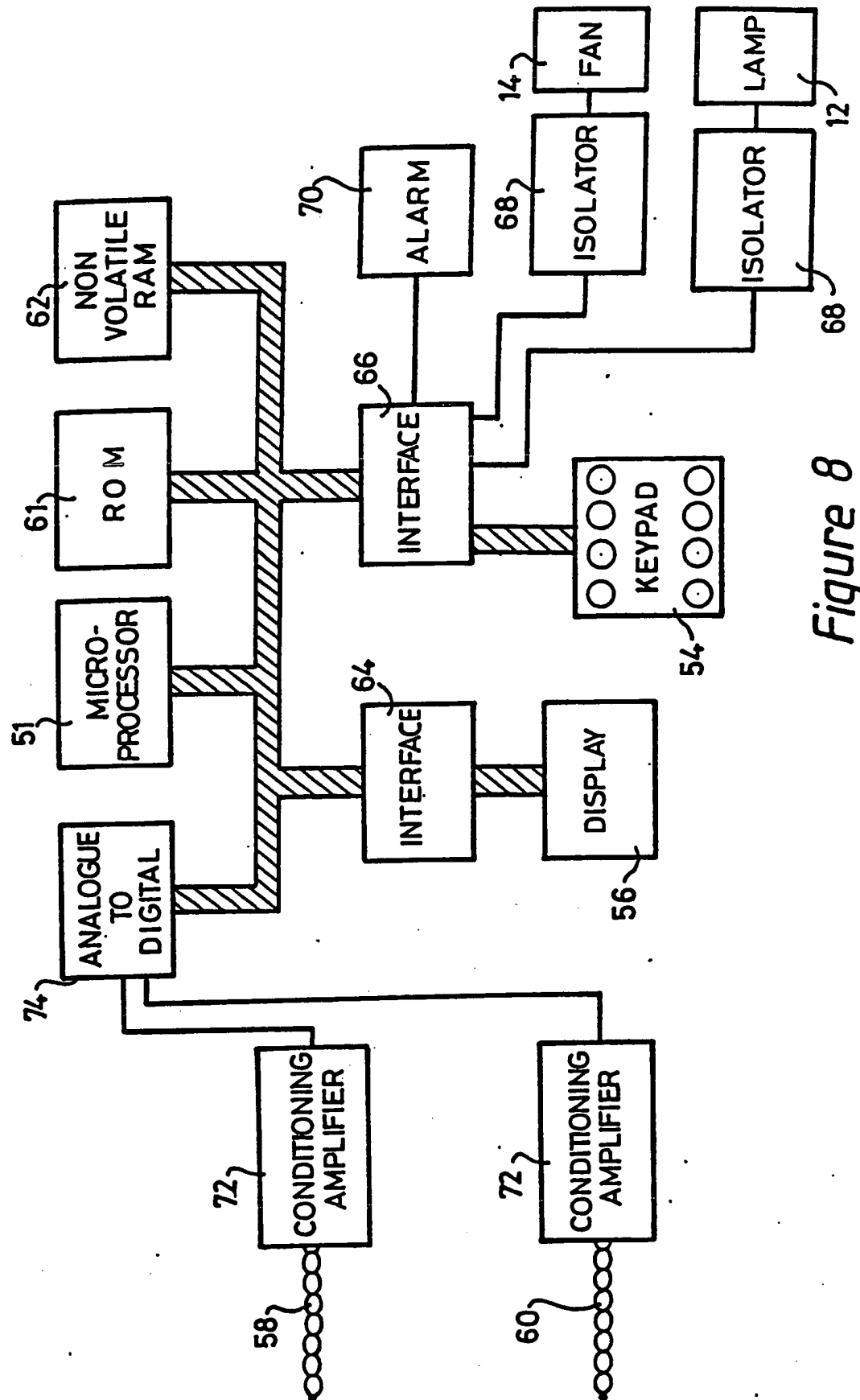


Figure 8

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 89/00323

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 4 G05D23/20 ; B01L7/02		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 4	G05D ; B01L	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	US,A,4429829 (E. DUTTON) 07 February 1984 see column 2, line 42 - column 3, line 20 see column 3, line 37 - column 4, line 33; figure 1	1-9, 12-14
Y	EP,A,0236069 (CETUS CORPORATION) 09 September 1987 see abstract see page 8, line 20 - page 9, line 24 see page 12, line 9 - line 27 see figures 1, 2	1-3, 12-14
Y	US,A,3634651 (M. SIEGEL) 11 January 1972 see column 3, line 58 - column 5, line 7; figures 1, 2, 4, 7	4
-/-		
<p>¹⁰ Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"R" earlier document but published on or after the international filing date</p> <p>"T" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"L" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
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